## **CLAIMS**

What is claimed is:

1	1. A method for determining the effect of a test agent on a tissue engineered			
2	cartilage matrix, comprising:			
3	(A) culturing an engineered cartilage tissue comprising the steps of:			
4	(i) culturing isolated chondrogenic cells for an amount of time effective			
5	for allowing formation of a chondrogenic cell-associated matrix; and			
4 F 8 8 9	(ii) culturing the chondrogenic cells with the cell-associated matrix on a			
	semipermeable membrane in the presence of a growth factor for a time effective for allowing			
	formation of the engineered cartilage tissue;			
<sup>1</sup> 9	(B) contacting one or more test agents with one or more cells or tissues selected			
<b>1</b> 0	from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the chondrogenic			
<b>占</b> 1 <b>占</b> 12	cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii), (d) the			
	chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage tissue;			
14 1113	and			
112 113 114	(C) measuring the effect the one or more test agents has on the contacted cells or			
15	tissue.			
1	2. The method of claim 1 wherein the chondrogenic cell-associated matrix			
2	comprises aggrecan, collagen types II, IX and XI, matrix proteins and hyaluronan.			
1	3. The method of claim 1 wherein the engineered cartilage tissue comprises			
2	collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc3 aggrecan,			
3	wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the			

ratio of aggrecan to collagen is about 1:1 to about 10:1.

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1	4.	The method of claim 1 wherein the isolated chondrogenic cells are from		
2	articular cartilage.			
1	5.	The method of claim 1 wherein the isolated chondrogenic cells are from		
2	costal cartilage, nasa	al cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thyroid		
3	3 cartilage, arytenoid cartilage or cricoid cartilage.			
1	6.	The method of claim 1 wherein the isolated chondrogenic cells are from		
2	fibrocartilage.			
1	7.	The method of claim 6 wherein the fibrocartilage is ligament, tendon,		
1 2 1 3	meniscus or intervertebral disc.			
THE THE TANK OF THE STATE OF TH	8.	The method of claim 1 wherein step (i) comprises culturing the		
¥ 2	chondrogenic cells	on an alginate medium.		
1 1	9.	The method of claim 1 wherein step (C) comprises measuring the amount		
1 2	of proteoglycan in the engineered cartilage tissue.			
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	10.	The method of claim 1 wherein step (C) is performed without the addition		
TU 2	of extrinsic radioact	civity.		
1	11.	The method of claim 10 wherein step (C) comprises enzymatically		
2	degrading the engineered cartilage tissue.			
1	12.	The method of claim 11 wherein step (C) further comprises staining the		
2	enzymatically degra	aded engineered cartilage tissue with a dye.		
1	13.	The method of claim 1 wherein the engineered cartilage tissue is removed		
2	from the semiperme	eable membrane prior to being contacted with the test agent.		

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1	14.	The method of claim 1 further comprising:		
2	(D) identifying one or more test agents that have desirable properties; and			
3	(E) producing the one or more test agents as a therapeutic drug.			
1	15. A	kit for determining the effect of a test agent on a tissue engineered cartilage		
2	matrix comprising instructions for carrying out the method of claim 1.			
1	16.	The kit of claim 15 further comprising one or more of:		
2		(i) one or more reagents;		
3		(ii) an enzyme capable of degrading the engineered cartilage tissue;		
4		(iii) a dye capable of labeling a component of the engineered cartilage		
<u>f</u> 5	tissue; and			
<b>D</b> 6		(iv) an antibody capable of labeling a component of the engineered		
	cartilage tissue.			
<u>.</u> 1	17.	A method for determining the effect of a test agent on a tissue engineered		
= 2 []	cartilage matrix, comprising:			
디 남 3	(A)	culturing an engineered cartilage tissue comprising the steps of:		
7U 711 4	,	(i) culturing isolated chondrogenic cells for an amount of time effective		
of the first said for the first said for the first said for the first said for the first for the fir	for allowing formation	on of a chondrogenic cell-associated matrix; and		
ГЦ 6	S	(ii) culturing the chondrogenic cells with the cell-associated matrix on a		
7	semipermeable membrane in the presence of a growth factor for a time effective for allowing			
8	formation of the engineered cartilage tissue;			
9	(B)	contacting one or more test agents with one or more cells or tissues		
10	selected from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the			
11	chondrogenic cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii),			
12	(d) the chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage			
13	tissue in the presence of a known modulator of cartilage tissue; and			
14	(C)	measuring the effect the one or more test agents has on the contacted cells		
15	or tissue.			

1 2	18.	The method of claim 17 wherein the chondrogenic cell-associated matrix, collagen types II, IX and XI, and hyaluronan.			
_	combines aggreem	, ••			
1	19.	The method of claim 17 wherein the isolated chondrogenic cells are from			
2	articular cartilage.				
1	20.	The method of claim 17 wherein the isolated chondrogenic cells are from			
2	<ul> <li>costal cartilage, nasal cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thyroi</li> <li>cartilage, arytenoid cartilage or cricoid cartilage.</li> </ul>				
3					
1	21.	The method of claim 17 wherein the isolated chondrogenic cells are from			
<u></u> 2	fibrocartilage.				
	22.	The method of claim 21 wherein the fibrocartilage is ligament, tendon,			
± 2	meniscus or interve	rtebral disc.			
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1= [] 1	23.	The method of claim 17 wherein step (i) comprises culturing the			
<u> </u>		• • • • • • • • • • • • • • • • • • • •			
[] Z	chondrogenic cells on an alginate medium.				
I off cont many first south	24.	The method of claim 17 wherein the engineered cartilage tissue comprises			
<u> </u>	collagen types II D				
	collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc <sup>3</sup> aggrecan,				
3	wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the				
4	ratio of aggrecan to	collagen is about 1:1 to about 10:1.			
1	25.	The method of claim 17 wherein step (C) comprises measuring the amount			
2	of proteoglycan in the engineered cartilage tissue.				

The method of claim 17 wherein step (C) is performed without the

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addition of extrinsic radioactivity.

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The method of claim 26 wherein step (C) comprises enzymatically 1 27. 2 degrading the engineered cartilage tissue. The method of claim 27 wherein step (C) further comprises staining the 28. 1 2 enzymatically degraded engineered cartilage tissue with a dye. The method of claim 17 wherein the modulator of the engineered cartilage 29. 1 tissue is a matrix stimulating agent, cytokine or TNF-α. 2 30. The method of claim 29 wherein the cytokine is interleukin-1. 1 <u>į.</u> 1 A kit for determining the effect of a test agent on an engineered cartilage 31. tissue comprising instructions for carrying out the method of claim 17. The kit of claim 31 further comprising one or more of: 32. (i) one or more reagents; (ii) an enzyme capable of degrading the engineered cartilage tissue; (iii) a dye capable of labeling a component of the engineered cartilage tissue; and (iv) an antibody capable of detecting a component of the engineered **N** 7 ivcartilage tissue. 1 The method of claim 17 further comprising: 33. 2 (D) identifying one or more test agents that have desirable properties; and 3 (E) producing the one or more test agents as a therapeutic drug.

The method of claim 17 further comprising removing the engineered

cartilage tissue from the semipermeable membrane prior to contacting the engineered cartilage

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tissue with the test agent.

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1 35. The method of claim 17 wherein steps (A) and (B) occur in the same well

2 of a multiwell plate.